Inhibition of the Key Glycolytic Enzyme PFKFB3 with Novel Compounds Suppresses Vessel Sprouting

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Aim
Intraplaque angiogenesis is an important contributor to atherosclerotic plaque growth and instability. Angiogenic signals induce endothelial cells (ECs) to switch their metabolism to being highly glycolytic, enabling their growth and division. Glycolytic modulation by inhibition of the glycolytic activator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) has been shown to reduce angiogenesis. The objective of this study was to identify novel anti-angiogenic compounds with a potential to efficiently modulate (inhibit) angiogenesis.

Methods
Using the human EC line EA.hy926, we studied the effects of PFKFB3 inhibition with 3PO, a weak competitive inhibitor of PFKFB3, and of two potent self-synthesized phenoxindazole analogues (PA-1 and PA-2) on glycolysis, proliferation, migration, matrix metalloproteinase (MMP) activity, and capillary tube formation. The latter experiment was also performed using primary human umbilical vein endothelial cells (HUVEC). Moreover, gene expression of important markers related to angiogenesis were measured at mRNA level by real-time PCR.

Results
PFKFB3 inhibition with all three tested compounds significantly reduced glycolytic activity. While PA-1 and PA-2 suppressed capillary tube formation in both cell groups, 3PO did not have any effect in EA.hy926 ECs and even an inducing effect in the HUVECs. Accordingly, PA-1 and PA-2 markedly inhibited EC migration, proliferation and wound closing capacity which are essential for neovessel formation. Moreover, these inhibitors downregulated gelatinase gene expression up to 6-fold, as well reduced the activity of proMMP-9 and MMP-2 up to 50% and 30% compared to control, respectively. Gene expression analysis revealed that the PA compounds downregulated PFKFB3 expression whilst 3PO did not. Similarly, markers of migration and angiogenesis, such as CCL5, VCAM-1, VEGFA and VEGFR2, were also markedly reduced (up to 10-fold) by the PA compounds.

Conclusions
These findings suggest that PFKFB3 inhibition with PA compounds may interfere with key pro-angiogenic functions, such as endothelial migration, proliferation and capillary-like structure formation and this exerts a multitarget anti-angiogenic activity. Hence, PFKFB3 inhibition with PA compounds is a promising therapeutic approach to promote plaque stability.